Screening and identification of salt-tolerant lines in novel chickpea (*Cicer arietinum* L.) germplasms

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Abstract
Salinity is the most serious abiotic stress for plants growth. In the present study, the response of eighteen novel chickpea genotypes was studied under salinity. Salt stress significantly ($p < 0.05$) reduces plant growth (shoot length, shoot weight, root length, root weight, etc.) and impacts physiological parameters (relative water content, membrane stability index, total chlorophyll content) in the selected genotypes. Based on the percentage reduction of the above-mentioned attributes, the experimental genotypes were divided into salt-sensitive and salt-tolerant categories. Genotypes IC326761, IC375927, IC223042, and IC269123 showed salt tolerance, while the genotypes IC327642 and IC326265 were considered as salt sensitive. Tolerant genotypes (IC326761, IC375927, IC223042, and IC269123) were able to maintain the maximum dry matter, membrane stability index, total chlorophyll content, and relative water content under saline conditions.

The results showed a significant correlation between salt tolerance and physiological properties of chickpea. Hence, these physiological properties could be used as a marker for the selection of salt-tolerant chickpea lines. These markers can significantly separate salt potential genotypes from a large number of germplasm sets at the germination stage using pot-culture which will help to a large extent in reducing labor costs during field trials. The current study has identified new donors for salinity tolerance that will further contribute to the development of high-yielding salt-tolerant chickpea varieties.

Keywords: Chickpea, Germplasm, Salinity, Tolerant.

Introduction

In agriculture, the biggest challenge of the twenty-first century is to fight the various biotic and abiotic stresses along with the increasing population. Among them, soil salinity is one of the major stress and has been identified as the second leading and fastest-spreading problem[48].

Right now, the total agricultural land affected by salinity is 6.74 million ha and by 2050 the area will increase up to 16.2 million ha[20]. Although the major staple food crops in the world are affected by the soil salinity, however, the essential nutrients present in the legume crops as compared to grains made an additional loss[22].

Chickpea comparatively contains plenty of protein which is 40% of its total weight and thus makes it exceptional among all pulse crops. It is an excellent source of energy as it contains minerals, vitamins, fiber and potentially health-beneficial phytochemicals. All these nutritional features present in chickpea make it affordable, popular, and economical especially in developing countries[24].

Currently, the land area used for chickpea cultivation globally is approximately 14.56 million hectares, which has increased comparatively from the last few decades[25].

It usually grows in semi-arid areas that are prone to soil salinity. Nevertheless, since the annual production loss is estimated to be 8% to 10%, it is placed in the very sensitive category of salinity[12].

In the view of the negative effects of salt stress on chickpea growth and productivity, identification and selection of salt-tolerant genetic resources have established significant interest from the past few decades. Salt tolerance has been confirmed by several physiological characters that have been observed in response to salinity stress to identify tolerance / sensitive genotypes[23]. Physiological factors analysis at the seedlings stage is the best possible way to select salt-tolerant genotypes[11].

Thus, considering the above description, the major objective of the current study was to identify salt-tolerant chickpea genotypes from a broad chickpea germplasm accession set and evaluate important morphological and physiological traits under salinity stress.

Material and Methods

Selection of Experimental material: In the present study total of 18 randomly selected chickpea germplasm accessions were evaluated under 80 mM level of salinity for assessment of germination percentage. CSG8962 (tolerant), JG11 (tolerant) and IC3230 (susceptible) genotypes were used as checks (Table 1). These accessions were obtained from the National Plant Genetic Resources (NBPGR), New Delhi, and Indian Institute of Pulses Research (IIPR), Kanpur.

Induction of salt stress in pots: Chickpea seeds were grown in plastic pots with a diameter of 8 cm and a soil weight of 0.4 kg under control and saline conditions.

Table 1
Details of chickpea germplasm accessions used in this study

S.N.	Accession number	Accession type	Country of origin	Source
1.	**CSG 8962**	Check (Tolerant)	India	IIPR, Kanpur
2.	**JG 11**	Check (Tolerant)	India	IIPR, Kanpur
3.	IC209670	Experimental material	India	NBPGR, New Delhi
4.	IC223042	Experimental material	India	NBPGR, New Delhi
5.	IC269123	Experimental material	India	NBPGR, New Delhi
6.	IC269629	Experimental material	India	NBPGR, New Delhi
7.	IC270861	Experimental material	India	NBPGR, New Delhi
8.	IC272471	Experimental material	India	NBPGR, New Delhi
9.	IC305561	Experimental material	India	NBPGR, New Delhi
10.	IC326265	Experimental material	India	NBPGR, New Delhi
11.	IC326761	Experimental material	India	NBPGR, New Delhi
12.	IC327642	Experimental material	India	NBPGR, New Delhi
13.	IC327777	Experimental material	India	NBPGR, New Delhi
14.	IC373447	Experimental material	India	NBPGR, New Delhi
15.	IC375927	Experimental material	India	NBPGR, New Delhi
16.	IC424807	Experimental material	India	NBPGR, New Delhi
17.	IC505314	Experimental material	India	NBPGR, New Delhi
18.	IC561350	Experimental material	India	NBPGR, New Delhi
19.	IC83462	Experimental material	India	NBPGR, New Delhi
20.	ICC2792	Experimental material	Iran	NBPGR, New Delhi
21.	**ICC3230**	Check (Susceptible)	Iran	NBPGR, New Delhi

The salinity stress was induced by treating normal soil (1.2 EC) of the pot with sodium chloride (NaCl) solution. NaCl solution was prepared by dissolving sodium chloride (NaCl) in double distilled water with a final concentration of 80 mM (8 EC). The treatment pots were watered with NaCl solution (80 mM), whereas the control pots were watered with double distilled water (0 mM). The salinity level was maintained throughout the experimental duration by regularly recording the electrical conductivity (EC) of the soil. The experiment was carried out under open environmental conditions and the soil moisture was maintained through the regular irrigation.

The plants were grown from the day of sowing to 30–40 days under salt-stressed conditions. The data of various morphological characteristics were recorded such as Shoot length (SL), Root length (RL), Fresh shoot weight (FSW), Dry shoot weight (DSW), Fresh root weight (FRW), Dry root weight (DRW), and Plant height (PH) while physiological parameters like membrane stability index (MSI)[10], total chlorophyll content, and relative water content (RWC)[8] were measured from the third leaf of the top of the control and salt-treated chickpea plant. Dimethyl sulfoxide (DMSO) was used to extract the total chlorophyll content from the fresh leaves (100 mg) according to the method of Hiscox and Israelstam.

Data analysis: The mean values of each trait were taken for statistical analysis. The variance analysis (ANOVA) was analyzed using SPSS statistics 19 (IBM Corp.) software. The statistical significance was calculated at a 5% level of significance. NTSYS-PC software (version 2.21b) was used for the clustering of genotypes using the euclidean dissimilarity coefficient.

Results and Discussion

The major objective of this study was to identify salt-tolerant chickpea genotypes from a broad chickpea germplasm accession set and evaluate important morphological and physiological traits under salinity stress. Various researchers have found significant morphological and physiological differences in chickpea under salinity stress[33, 44, 45]. In this study, 18 genotypes and three checks were evaluated for SL, RL, FRW, DRW, FSW and DSW and physiological parameters RWC, MSI, and TCC.

Shoot and Root length: The roots of the plant are in direct contact with the soil. They absorb water from the soil and then supply it to the shoot for the rest parts of the plant. Therefore, the lengths of the roots and shoots are the most important parameters to analyze salt stress and provide important clues to know the plant's response to salt stress[17].

Under the salt-stressed condition, SL varied from 8.75 to 25.45 cm, while RL varied from 0.25 to 18.55 cm. The highest RL and SL were found in genotype IC326761 (15.92 and 25.45 cm) after checks JG11 and CSG8962, at 80 mM (Table 2). The lowest SL and RL were found in genotype IC327642 (8.75 and 0.25 cm). The percentage reduction of SL and RL for different genotypes ranged from 1.23% to 58.43% and 1.42% to 89.8% respectively (Table 3). The RL of genotype IC326761 decreased the least (1.42%), while the SL decreased by 1.61% after checks JG11 (1.55%) and CSG8962 (1.23%).

In this study, it was observed that the length of plant roots and shoots decreased under salt stress. The reduction of plant growth by salinity may be due to the inhibitory effect of ions. It causes an imbalance in the nutrient and water absorption of seedlings[46]. Neumann[31] pointed out that salinity inhibits root growth, so the ability of plants to absorb water and essential mineral nutrients from the soil reduces. In the present study it was observed that the inhibitory effect of salt stress adversely affected root growth than shoot. These findings are consistent with Kaya et al[20] and Misra and Dwivedi.[27] It was also reported that with the increase of salinity, the dry root weight of chickpeas significantly decreases[26,29].

Fresh and Dry weight of Shoots and Roots: With higher salt concentrations, the effect of salt stress on chickpea seedling becomes apparent. The minimum and maximum values of FSW were found to be 0.51 to 0.62 g with an average value of 0.53 g under normal and 0.09 g to 0.58 g in salt-stressed with an average weight of 0.31 g. Under salt stress conditions, the dry shoot weight (DSW) showed a small change, ranging from 0.01 to 0.06 g with an average of 0.03 g. Similarly, the minimum and maximum value fresh root weights (FRW) were found to be 0.13 to 0.48 g with an average value of 0.25 gm whereas dry root weight (DRW) ranged from 0.01 to 0.04 g with a mean of 0.02 g under salinity condition.

The percentage reduction of FSW and DSW for different genotypes ranged from 6.43% to 82.35% and 7.29% to 80.72% respectively whereas the percentage reduction of FRW and DRW of different genotypes varied from 7.46% to 60.61% and 11.22% to 61.86% respectively. Under consideration of the entire chickpea germplasm used in present study, genotype IC326761 was least affected by salt compared to the control. Besides this, genotypes IC375927, IC223042, and IC269123 were moderately affected by salt.

In the present study, a significant reduction has been found in the fresh and dry weight of chickpea roots and shoots. The results indicate that a lot of physiological variation occurs in chickpea germplasm under salinity and are consistent with Amirjani[2].

In addition, Tatar et al[42] also observed a significant decrease in the total dry mass of seedlings in rice varieties under salt stress. Similarly, Senadheera et al[35] reported that at the seedling stage, salt stress (50 mM NaCl) caused a significant reduction in FW and DW of salt-sensitive lines IR29. The reduction of plant biomass under salinity may be due to ion toxicity, disturbed metabolic pathways, and loss of turgor.

Table 2
Mean value of different traits at control and salinity stress condition

Variety	SL (cm)		RL (cm)		FSW (g)		DSW (g)		FRW (g)		DRW (g)		MSI (%)		RWC (%)		TCC (mg/g FW)	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
CSG 8962	24.45	24.15	18.05	17.55	0.59	0.55	0.05	0.05	0.49	0.44	0.05	0.04	59.97	56.45	61.37	60.99	7.30	6.90
JG11	25.85	25.45	18.95	18.55	0.62	0.58	0.06	0.06	0.52	0.48	0.05	0.04	63.65	60.56	62.12	61.67	8.60	8.30
IC209670	25.70	15.90	2.30	0.70	0.51	0.15	0.03	0.01	0.39	0.16	0.03	0.01	57.54	34.19	54.17	32.08	5.20	1.30
IC223042	20.75	19.55	16.25	14.13	0.52	0.44	0.05	0.04	0.42	0.35	0.03	0.02	58.43	54.31	58.24	55.96	7.20	6.30
IC269123	20.05	18.95	15.65	14.05	0.53	0.46	0.04	0.04	0.45	0.38	0.03	0.02	59.35	54.33	57.32	54.41	7.10	6.40
IC269629	25.05	14.75	3.75	1.25	0.55	0.17	0.07	0.01	0.38	0.17	0.03	0.01	57.33	31.47	53.21	23.90	4.90	3.10
IC270861	23.90	19.43	11.61	7.13	0.52	0.41	0.03	0.03	0.39	0.25	0.02	0.01	54.76	46.41	58.11	51.94	6.80	5.30
IC272471	24.95	12.75	4.45	1.66	0.53	0.11	0.06	0.01	0.43	0.17	0.02	0.01	56.32	29.33	55.23	23.96	4.80	2.40
IC305561	21.90	13.40	2.30	0.50	0.50	0.15	0.06	0.02	0.41	0.18	0.03	0.01	58.44	36.05	56.11	31.98	5.30	2.50
IC326265	24.15	11.75	2.45	0.35	0.52	0.13	0.04	0.01	0.32	0.14	0.02	0.01	55.63	19.56	54.31	16.61	4.60	1.70
IC326761	24.85	24.45	16.15	15.92	0.62	0.57	0.06	0.06	0.46	0.42	0.05	0.04	61.71	58.82	61.33	60.98	7.50	7.10
IC327642	21.05	8.75	2.45	0.25	0.51	0.09	0.04	0.01	0.33	0.13	0.03	0.01	54.88	20.74	55.32	14.05	4.50	1.90
IC327777	23.90	19.79	10.76	6.34	0.52	0.41	0.04	0.03	0.39	0.26	0.02	0.01	56.54	46.62	59.13	51.41	6.70	5.50
IC373447	21.10	11.90	3.30	1.20	0.49	0.12	0.05	0.01	0.38	0.21	0.02	0.01	59.63	37.78	54.45	40.45	5.60	3.50
IC375927	23.25	22.75	15.45	14.95	0.56	0.51	0.05	0.04	0.42	0.37	0.03	0.03	56.15	52.84	59.93	59.18	7.20	6.60
IC424807	22.30	17.44	9.28	5.92	0.54	0.45	0.05	0.04	0.34	0.23	0.02	0.01	57.23	46.59	57.22	52.13	6.80	5.60
IC505314	26.50	17.20	4.80	1.90	0.48	0.31	0.04	0.02	0.35	0.19	0.03	0.01	59.82	37.56	55.32	40.17	5.10	2.70
IC561350	24.05	13.55	3.15	0.85	0.51	0.12	0.07	0.01	0.36	0.18	0.02	0.01	56.27	27.72	57.27	24.10	4.90	2.20
IC83462	20.70	16.52	7.83	4.63	0.54	0.41	0.05	0.04	0.37	0.25	0.02	0.01	55.44	44.41	59.25	51.71	6.60	5.40
ICC2792	24.30	15.60	3.80	1.14	0.53	0.14	0.04	0.02	0.41	0.17	0.02	0.01	58.67	35.19	52.14	32.07	5.50	2.20
ICC3230	27.95	17.35	5.15	1.95	0.49	0.31	0.06	0.01	0.37	0.15	0.03	0.01	55.97	25.25	56.28	19.27	4.70	2.60

Because of these factors, the function of the gas exchange properties got disturbed which ultimately leads to a decrease the photosynthesis activity[38]. Therefore, the decrease in plant biomass in this study may be due to decreased photosynthesis.

Total Chlorophyll Content: The total chlorophyll content is considered to be an important parameter of crop salt tolerance[16]. Salt stress causes leaf chlorosis, which ultimately leads to photoinhibition and photodestruction of chlorophyll pigments and thus has a negative effect on plant chlorophyll content.

In the present study, chickpea genotypes showed a fall in total chlorophyll content under salt stress. Under normal conditions, TCC varied from 4.6 to 8.6 mg/g with an average value of 6.04 mg/g, but under stress conditions, it varies from 1.7 to 8.3 mg/g with an average value of 4.26 mg/g (Table 2). It was observed that IC326761 (5.33%), JG11 (3.49%), and CSG8962 (5.48%) showed the minimum drop in chlorophyll content under salt stress, while IC209670 showed the maximum drop (75%) in chlorophyll content. Similar results were reported by various researchers in other legumes crops[1,4,38,40]. Low degradation of chlorophyll content in some chickpea germplasm under salinity stress indicates their better photosynthetic capacity.

Thus, chlorophyll content can be used as a reliable biochemical marker of plant salt tolerance[4,39]. Genotype IC326761 maintains the maximum total chlorophyll content. A combined effect of the following physiological parameters viz. low stomatal conductance, reduction in carbon absorption and metabolism, loss of photochemical ability may cause the inhibitory effect of TCC under salt stress[4,30].

Beltagi[9] observed the loss of photosynthesis up to 60% in chickpea genotypes grown under salt stress. The loss of chlorophyll pigment may be due to the increase of chlorophyllase activity and due to the inhibition of specific enzymes responsible for the synthesis of green pigment[3,27].

Membrane Stability Index: Electrolyte leakage is associated with the leakage of solute from the cell and is commonly used as a standard to assess the level of membrane stability[7]. Since membrane damage increases with increasing salinity levels, MSI is considered an important tool to evaluate the salt tolerance potential of chickpea genotypes[38].

At normal conditions, the membrane stability index (MSI) ranged from 54.88 to 63.65%, with an average value of 57.80%, while in salt-stressed conditions, the membrane stability index varied from 19.56 to 60.56% with an average value of 40.77%. The salt tolerance check genotypes CSG8962 (56.45%) and JG11 (60.56%) had significantly higher MSI as compared to susceptible check ICC3230 (25.25%) (Table 3). The outcome of this study showed that IC326761 (58.82%) maintained higher membrane stability than others.

These results are consistent with the results reported by Sairam et al[34] and Garg and Singla,[13] among which the tolerant genotype of wheat and chickpea respectively has a higher MSI value.

Table 3
Percentage reduction of various morphological and physiological traits at salinity stress condition

Variety	MSI	RWC	TCC	DRW	FRW	DSW	FSW	RL	SL	Overall reduction
CSG 8962	5.87	0.64	5.48	13.2	9.86	8.38	6.43	2.77	1.23	5.98
JG11	4.85	0.72	3.49	11.22	7.46	7.29	6.89	2.11	1.55	5.06
IC209670	40.58	40.78	75	61.86	58.97	59.73	70.59	69.57	38.13	57.25
IC223042	7.05	3.91	12.5	20.48	18.16	19.73	14.17	13.05	5.78	12.76
IC269123	8.46	5.08	9.86	18.9	16.29	14.29	13.67	10.22	5.49	11.36
IC269629	45.11	55.08	36.73	59.71	55.26	80.03	69.09	66.67	41.12	56.53
IC270861	15.25	10.62	22.06	39.73	36.13	19.65	19.61	38.59	18.7	24.48
IC272471	47.92	56.62	50	47.93	60.47	79.68	79.25	62.7	48.9	59.27
IC305561	38.31	43	52.83	58.93	56.1	70.07	70	78.26	38.81	56.26
IC326265	64.84	69.42	63.04	47.95	56.25	67.49	75	85.71	51.35	64.56
IC326761	4.68	0.57	5.33	14.29	8.84	8.41	6.97	1.42	1.61	5.79
IC327642	62.21	74.6	57.78	59.71	60.61	73.76	82.35	89.8	58.43	68.81
IC327777	17.55	13.06	17.91	36.68	31.69	26.9	22.37	41.08	17.2	24.94
IC373447	36.64	25.71	37.5	52.32	44.74	78.17	75.51	63.64	43.6	50.87
IC375927	5.89	1.25	8.33	19.12	13	10.69	8.72	3.24	2.15	8.04
IC424807	18.59	8.9	17.65	32.26	32.85	26.63	17.68	36.21	21.79	23.62
IC505314	37.21	27.39	47.06	56.23	45.71	54.87	35.42	60.42	35.09	44.38
IC561350	50.74	57.92	55.1	51.27	50	80.72	76.47	73.02	43.66	59.88
IC83462	19.9	12.73	18.18	34.78	32.97	25.56	22.91	40.87	20.19	25.34
ICC2792	40.02	38.49	60	51.49	58.54	61.14	73.58	70	35.8	54.34
ICC3230	54.89	65.76	44.68	57.82	59.46	78.07	36.73	62.14	37.92	55.27

Table 4
Variance analysis (ANOVA) for different traits under salinity stress

		Sum of Squares	Mean Square	F	Sig.	% Variation
SL	Between Groups	435.99	435.99	35.00	0.000	46.67
	Within Groups	498.27	12.46			53.33
RL	Between Groups	564.37	564.37	18.71	0.000	31.87
	Within Groups	1206.44	30.16			68.13
FSW	Between Groups	0.50	0.50	30.42	0.000	43.20
	Within Groups	0.66	0.02			56.80
DSW	Between Groups	0.01	0.01	29.35	0.000	42.32
	Within Groups	0.01	0.00			57.68
FRW	Between Groups	0.23	0.23	31.60	0.000	44.14
	Within Groups	0.29	0.01			55.86
DRW	Between Groups	0.00	0.00	11.64	0.001	22.53
	Within Groups	0.00	0.00			77.47
MSI	Between Groups	3043.86	3043.86	36.99	0.000	48.05
	Within Groups	3291.37	82.28			51.95
RWC	Between Groups	2733.15	2733.15	19.93	0.000	33.26
	Within Groups	5485.07	137.13			66.74
TCC	Between Groups	33.30	33.30	10.80	0.002	21.26
	Within Groups	123.32	3.08			78.74

Under stress conditions, plant species respond differently due to changes in their antioxidant systems, which can lead to oxidative damage and increase membrane permeability[34].

Relative Water Content: In this study, the relative moisture content (RWC) showed significant differences. Under normal circumstances, the RWC changed from 54.31% to 62.12% with an average of 57.04%; under salinity conditions, it ranged from 16.61 to 61.67 % and the average was 40.91%. IC326761 maintained a maximum of 60.98% RWC as compared to check JG11 (61.97%) and CSG8962 (60.99%). Percent reduction of RWC for different genotypes ranged from 0.57% to 74.6%.

Genotype IC326761 was able to maintain a minimum percentage reduction (0.57%) of RWC compared to check CSG8962 (0.64%). The present study showed that under high salinity stress the RWC of chickpea decreases. Our results are in coherence with the report on other legumes viz. alfalfa[37] and mung bean[19,31].

Suriya-arunruj et al[41] reported similar results in rice. High soil salinity causes low substrate water potential and damage to the root system, resulting the reduction in water absorption rate and hence reduced RWC. The adverse effect on plant RWC is caused by the increase of soluble salt. It inhibits the absorption of water and nutrients which leads to osmotic effects and toxicity[18,47].

Cluster and Variance analysis (ANOVA): A one-way analysis of variance (ANOVA) revealed significant ($p < 0.05$) differences among the 18 novel chickpea germplasms under salt stress (Table 4). A considerable variation >75% was found within the population while >45% was found between the population for all traits.

The highest percentage of variation between populations was observed for MSI (48.05%) while the least was observed in TCC (21.26%).

The quantitative information was analyzed by the Euclidean distance matrix and genotype grouped by SAHN. The dendrogram divided the genotypes into three different groups. In this study, the high tolerance check lines CSG8962 and JG11 and the 4 novel genotypes clustered together and formed a unique group I (Figure 1).

Four genotypes with moderate tolerance fall under group II. And the highly susceptible check genotype ICC3230 and 10 novel genotypes collectively formed a distinct group III. The newly identified highly tolerance lines present in the group I can be used as salt-tolerant donors.

Conclusion

In the present study, based on the morphological and physiological traits under salt stress, the distinctiveness of the experimental genotypes was characterized and it was found that the analysis of chickpeas at the seedling stage can be a promising approach to identify the salt-tolerant chickpea genotypes. Compared with the control conditions, all the parameters studied under the salt stress showed significant differences and overall reduction.

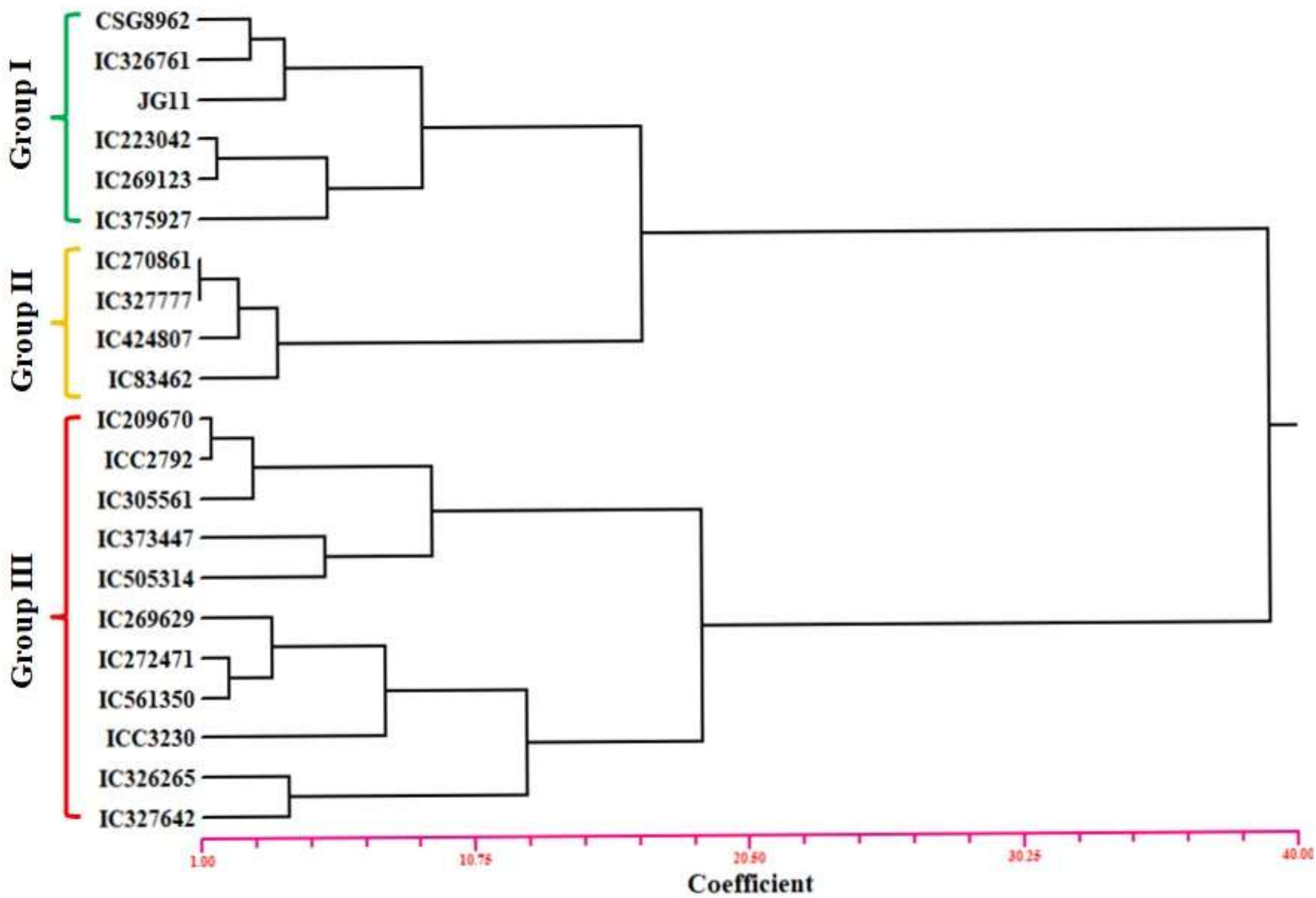

Figure 1: Grouping of chickpea germplasms based on SAHN under salinity stress condition

In this study, it was found that the germplasm with the least chlorophyll degradation had the least decrease in RWC. This indicates that these genotypes significantly synthesize photosynthetic compounds under stress conditions. Therefore, the physiological characteristics have proven to be very handy and useful, especially the shoot and root length, which have shown a regular drop in the growth under salt stress.

It could be concluded that relative water content, total chlorophyll content, plant growth, and membrane stability play an important role in the adaptation of chickpea genotype. Following genotypes IC326761, IC375927, IC223042, and IC269123 have been found highly tolerant with lower percentage reduction in MSI level, chlorophyll content, and relative water content than others. The results showed a significant correlation between salt tolerance and physiological properties of chickpea. Hence, these physiological properties could be used as a marker for the selection of salt-tolerant chickpea lines.

The tolerant genotypes identified in present study can be considered as donors for salinity traits and contributed to the development of high yielding salt-tolerant chickpea varieties. This study significantly separates salt potential genotypes at the germination stage using pot-culture, which will help to a large extent in reducing labor costs during field trials.

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